Prominence of *NUDT15* genetic variation associated with 6-mercaptopurine tolerance in a genome-wide association study of Japanese children with acute lymphoblastic leukemia

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Summary

Inherited genetic variation is associated with 6-mercaptopurine (6-MP) dose reduction and frequent 6-MP induced toxicities. However, tolerable dose for 6-MP is not completely predicted by the known variation in *NUDT15* and *TPMT* among Asian children with acute lymphoblastic leukemia (ALL). We performed a genome-wide association study (GWAS) related to 6-MP dose among Japanese children with ALL. This GWAS comprised 224 patients previously enrolled in Tokyo Children's Cancer Study Group clinical studies with replication attempted in 55 patients. Genome-wide single nucleotide polymorphism (SNP) genotypes were evaluated for association with 6-MP average dose during initial 168 days of maintenance therapy. Possible associations were observed across 5 gene coding regions, among which only variants at 13q14.2 were genome-wide significant and replicated (rs116855232, *NUDT15*, β=-10.99, P=3.7×10^{-13}). Notable findings were observed for variants in *AFF3* (rs75364948, P=2.05×10^{-6}) and *CHST11* (rs1148407, P=2.09×10^{-6}), but were not replicated possibly due to small numbers. A previously reported candidate SNP in *MTHFR* was associated with higher 6-MP average dose (rs1801133, P=0.045), and *FOLH1* (rs12574928) was associated in a candidate region evaluation (P_{adjust}=0.013). This study provides strong evidence that rs116855232 in *NUDT15* is the prominent genetic factor associated with 6-MP tolerable dose in Japanese.

Introduction

A survival probability of 80-90% in children with acute lymphoblastic leukemia (ALL) has been achieved due largely in part to advances in combination chemotherapy[1]. 6-mercaptopurine (6-MP) is a main component for improving therapeutic outcomes, but tolerability is different in each patient. Failure to minimize incidents of therapy interruption may affect prognostic outcomes for childhood ALL patients[2]. Response to 6-MP has been associated with variants in genes participating in the 6-MP metabolism pathway[3]. It is well known that genetic variation of the *TPMT* gene is associated with 6-MP intolerance[3]. However, the *TPMT* variant was not shown to be associated with thiopurine induced toxicities in Japanese[4], and the frequencies of TPMT poor metabolizer was lower in East Asians compared with other races. In 2014, the *NUDT15* rs116855232 variant was reported to influence 6-MP intolerance identified through a genome-wide association study (GWAS), and the frequency of that variant is higher in Asians compared to populations of European and African ancestries[5]. Subsequently, *NUDT15* genotype was shown to affect 6-MP tolerability among Asians undergoing childhood ALL therapy[4].

To date, several candidate gene studies have shown that genetic variation in *NUDT15* is associated with 6-MP dose reduction and frequent 6-MP-induced toxicities in childhood ALL[4,6–13]. NUDT15 enzyme dephosphorylates thio-guanosine triphosphate (GTP) and deoxy thio-GTP to thio-guanosine monophosphate (GMP) and deoxy thio-GMP, respectively. Lower NUDT15 enzyme leads to increased thioguanosine incorporation ratio to 6-MP dose and 6-MP related toxicities. In some instances, severe toxicities requiring significant 6-MP dose reduction have been observed in patients who do not carry the known genetic risk factors for 6-MP tolerability. Thus, tolerable dose for 6-MP is not completely predicted by the variation in *NUDT15* and *TPMT*.

The aim of this study was to perform a GWAS to examine the role of inherited genetic variation related to 6-MP dose, with objectives to both identify newly associated variation, as well as to

characterize the variants previously reported in diverse populations, among Japanese childhood ALL patients. Moreover, we pursued a targeted examination of genetic variation across the candidate gene regions to identify additional associations with 6-MP dose.

Methods

Patients and sample collection

This GWAS comprised patients previously enrolled in a Tokyo Children's Cancer Study Group (TCCSG) clinical study including L89-12[14,15], L95-14[15,16], L99-15[17] and L04-16[18] from 23 clinical centers collaborating on this genomic study. Patients were recruited at the time of routine outpatient follow-up visit between 2013 and 2015, as previously described[19]. Briefly, patients were considered eligible if they were aged 19 years or younger at the time of ALL diagnosis and self-identified as Japanese. Saliva samples were collected at the time of follow-up visit after remission. DNA was extracted using the Oragene prepIT DNA extraction kit (DNA Genotek, Ottawa, Canada), and stored at -80 °C. The study protocol was approved by the institutional review boards of all collaborating institute and hospital involved in patient recruitment. Written informed consent was obtained from the parents of each participant together with a written informed consent or assent by the child depend on age.

Genotyping and quality control

Whole genome single nucleotide polymorphism (SNP) microarray genotyping was performed using the Illumina HumanCoreExome-12 v1.1 BeadChip (San Diego, CA) and has been described previously [19]. Briefly, quality control (QC) procedures comprised excluding samples if the genotype call-rate was below 95% and samples that exhibited relatedness based on an identity-by-descent analysis. SNPs were excluded if genotype call-rate was less than 99%, genotype distribution deviated

109 from that expected based on Hardy-Weinberg equilibrium ($P > 1 \times 10^{-6}$), or the minor allele frequency
110 (MAF) was less than 0.01. Additionally, principal components (PC) analysis based on a subset of the
111 post-QC genome-wide SNPs in low linkage disequilibrium (LD) was performed using EIGENSTRAT
112 2.0 software together with HapMap data from Japanese, and sample outliers were excluded based on
113 a plot of the leading PCs.
114 Genome-wide SNP imputation was performed using ShapeIT2 and Minimac4 with reference
115 population from the 1000 Genomes Project Phase III Version 5. SNP imputation QC comprised
116 excluding poorly imputed SNPs defined by an R^2 of less than 0.5, resulting in a total of 6,236,137
117 SNPs available for analysis.
118
119 Clinical data
120 The clinical data and details of the administration of 6-MP doses during maintenance therapy were
121 available for 289 Japanese patients. In TCCSG protocols, maintenance therapy is initiated with 40
122 mg/m^2/day of 6-MP and 25 mg/m^2/week of oral methotrexate. These dosages were adjusted to
123 maintain the target leucocyte count at 1,500 – 3,000/mm^3. We excluded patients who started at more
124 than 125% or less than 75% of protocol 6-MP dose, as therapeutic dose for 6-MP and MTX were
125 adjusted for drug induced toxicities before initiation of maintenance therapy. In total, we included
126 224 ALL patients (discovery cohort) who started maintenance therapy using the normal protocol
127 dose (30–50 mg/m^2/day). The outcome variable for this GWAS was defined as 6-MP average dose
128 for initial 168 days of maintenance therapy.
129
130 Replication series
131 The replication series included 55 patients who started maintenance therapy by normal protocol
132 recruited previously through TCCSG as part of a separate study[4]. DNA were extracted from peripheral

blood obtained at remission, and SNP genotyping was performed using Taqman real-time PCR assays (Applied Biosystems, Waltham, MA).

Statistical analysis

We performed genome-wide association analyses of SNPs in relation to 6-MP average dose for initial 168 days of maintenance therapy among the discovery cohort using linear regression assuming a log-additive genetic model of inheritance and adjusting for age at diagnosis. Association analysis assuming dominant and recessive genetic inheritance models were also performed. Results showing a $P < 5 \times 10^{-8}$ were considered statistically significance at the genome-wide level, and a $P < 1 \times 10^{-5}$ was considered as showing a suggestive association. In the replication series, association analysis was conducted similarly using linear regression assuming a log-additive genetic model and adjusting for age at diagnosis. We defined a Bonferroni corrected $P < 0.05$ as statistically significant in the replication. Additionally, among the top SNPs, the difference of 6-MP average dose across the three possible genotypes was evaluated using the Kruskal-Wallis test. Normality test of 6-MP distribution was evaluated using the D'Agostino-Pearson test.

The association of specific candidate SNPs reported previously, as well as the variants in coding regions of those candidate genes were examined. Association results with a nominal p-value of less than 0.05 was considered statistically significant for specific candidate SNPs. For regional examination of association results across candidate genes, SNPs with a nominal p-value of less than 0.05 were considered noteworthy, and a condensed list of SNPs pruned on LD ($r^2 > 0.50$) within each gene were adjusted for multiple testing based on 10,000 permutation of the data on the 6-MP dose outcome in which a p-value below a family-wise type I error rate threshold of 0.05 was considered statistically significant. Statistical analyses were performed using PLINK version 1.9, R software (version 3.6.1) and Prism 9 (GraphPad Software, San Diego, CA).

157

158 **Results**

159 Genome-wide association analysis of 6-MP average dose during 168 days of maintenance therapy

160 A total of 224 patients were included in the genome-wide association analysis. No patients
161 needed to be excluded due to outlying genetic ancestry based on PC analysis evaluations. The
162 characteristics of patients were shown in Table 1. Age at diagnosis was significantly correlated with
163 6-MP average dose (β = -0.88, $P = 6.96 \times 10^{-5}$). The median 6-MP average doses for the initial 168
164 days were 41.1 (interquartile range: IQR 25% – 75%, 32.8 – 48.6), and did not appear to deviate from
165 a normal distribution (P > 0.05).

166 In the genome-wide analysis of the discovery cohort, linear regression of the 6-MP average
167 dose adjusted for age at diagnosis showed minimal evidence of genomic inflation (λ =1.01)
168 (Supplementary Figure S1). The Manhattan plot of the results showed potential association of variants
169 representing 5 genetic coding regions ($P < 1 \times 10^{-5}$) with 6-MP average dose (Figure 1, Table 2 and
170 Supplementary Table S1). Genome-wide significant associations with 6-MP average dose were
171 observed for variants at the chromosome 13q14.2 region in which the leading SNP was rs116855232
172 located in *NUDT15* SNP ($\beta = -11.45$, $P_{additive} = 8.5 \times 10^{-10}$, $P_{dominant} = 2.27 \times 10^{-9}$). Other variants
173 within this cluster of associated SNPs were in LD with this SNP (Supplementary Figure S2, $r^2 > 0.8$).
174 Suggestive associations were observed at chromosome 2q11.2 (rs75364948, *AFF3*), 2p21
175 (rs14452634, *THADA*), 12q23.3 (rs1148407, *CHST11*) and 16q23.2-3 (rs12934986 and rs10153053,
176 *CMIP*) (Table 2). In a sensitivity analysis, the results excluding outlier values of 6-MP (defined as the
177 values of more than the third IQR + 1.5 × IQR or less than the first IQR + 1.5 × IQR) showed a
178 persistent association with the chromosome 13q14.2 region, and slight attenuation of association for
179 the other loci (Supplementary Figure S3).

180 To identify associations in the absence of the effect of the well-known loci for 6-MP intolerance,

we excluded patients who were carriers of the *TPMT* rs1142345 and *NUDT15* rs116855232 variants. In this cohort, 7 and 41 patients were carriers of the *TPMT* and *NUDT15* variants, respectively. Only one patient carried both the *TPMT* and *NUDT15* variants. Among the remaining 178 patients, the genome-wide analysis of 6-MP average dose showed potential associations with variants representing 13 genetic coding regions ($P < 1\times 10^{-5}$) (Supplementary Table S2). The results of the suggestive variants for *CHST11*, *CMIP* and *THADA* were similar to that of the full analysis.

Replication analysis

For the replication series, we selected three potentially associated SNPs that reside within genes that have been previously implicated in 6-MP tolerability or progression of hematological malignancy (Table 2). The association with *NUDT15* rs116855232 strongly replicated in this series ($P = 5.43 \times 10^{-5}$); however, while the direction of association was consistent, rs75364948 (*AFF3*) and rs1148407 (*CHST11*) were not significantly associated with 6-MP average dose (Table 2). Combining the estimates from the discovery and replication cohorts, a strong genome-wide significant association between rs116855232 (*NUDT15*) and 6-MP average dose was observed ($\beta = -10.99$, $P = 3.66 \times 10^{-13}$).

Variant rs116855232 in the *NUDT15* gene represents the prominent association with 6-MP average dose within our Japanese study. Among the combined discovery and replication cohorts, 51 patients were heterozygous for rs116855232 (C/T), 5 patients were homozygous for the variant (T/T). The median of the 6-MP average dose for patients with CT and TT were 31.6 and 13.1 mg/m^2/day, respectively; these 6-MP doses were significantly lower compared to that of patients with the CC genotype (41.9 mg/m^2/day, $P = 3.74 \times 10^{-11}$, Figure 2A).

Previously reported candidate genes and 6-MP tolerable dose

As a secondary aim, we selected candidate loci based on review of the literature and the

PharmGKB database (https://www.pharmgkb.org) and performed targeted examination of these candidate regions. SNPs rs1142345 (*TPMT)*[5], rs3765534 (*ABCC4)*[20], rs1142345 (*ITPA*)[21,22], rs4149056 (*SLCO1B1)*[23], rs1801133 (*MTHFR)*[24], rs61886492 (*FOLH1*)[25], rs12199316 (*NHLRC1*)[26] and rs72846714 (*NT5C2*)[27] have been studied previously as candidate loci for 6-MP tolerable dose and/or toxicities. Among these, *MTHFR* rs1801133 showed a significant association with 6-MP average dose in our study ($\beta = 2.26$, $P = 0.045$). However, the other reported genetic variants were not related with 6-MP dose in this analysis (Table 3). In addition, other variants in LD with specific candidate SNPs were not associated. A range of genetic variation across these candidate genes were targeted to examine whether other SNPs may be associated with 6-MP average dose in Japanese. SNP associations showing nominal p-values of less than 0.05 were observed for *MTHFR*, *FOLH1*, *ABCC4*, and *ITPA* (Table 3). Among these, rs12574928 in *FOLH1* showed a statistically significant association with 6-MP average dose after adjusting for multiple testing ($\beta = -8.02$, $P = 0.013$).

Discussion

We observed strong evidence showing that genetic variation at the chromosome 13q14.2 region is significantly associated with 6-MP average dose for initial 168 days of maintenance therapy. Among the cluster of association signals, *NUDT15* rs116855232 was the leading SNP, and the other variants spanning the *SUCLA2* and *MED4* genes were in strong LD with this SNP. *NUDT15* genetic variation has been shown to effect thiopurine tolerance in our previous studies, as well as others from Asian countries[4,12,28] In this study, 19 of 279 patients experienced a reduction of average 6-MP dose during the 168 days to a level less than 20 mg/m^2/day. Five of 19 patients (26%) who needed a dose reduction were carriers of the rs116855232 variant. Lower NUDT15 enzyme leads to increased thioguanosine incorporation ratio to 6-MP dose and 6-MP related toxicities. Based on the characteristics of the

current study comprising an ethnically homogeneous group of Japanese children with ALL, *NUDT15* was observed to have the strongest effect on 6-MP tolerance, and the associated variant rs116855232 is more frequent in Asians than other races/ethnicities.

The *TPMT* variant is a well-known genetic risk factor for 6-MP tolerance[3]. In this population, only 10 patients (1.8%) carried the *TPMT* rs1142345 variant, and their 6-MP average dose was 40.0 (19.1 – 57.4) mg/m^2/day. The MAF of rs1142345 in this Japanese cohort was lower than populations of European and African ancestry (MAF; 4 – 6%), and there were no patients who were homozygous carriers. While not significant possibly due to limitations in statistical power, the effect size of this variant was also weaker compared to reports from other races/ethnicities.

Interestingly, 2 patients with *TPMT* rs1142345 requiring 6-MP average dose of less than 20 mg/m^2, were carriers of the *NUDT15* rs116855232 variant. Yang et al. reported that patients with heterozygous genotypes for both *TPMT* rs1142345 and *NUDT15* rs116855232 had 30-60% of 6-MP standard dose during 6-months of maintenance therapy among the multi-ethnic patient cohort[5]. Choi et al. reported *NUDT15* rs116855232 was significantly associated with high 6-thioguanine nucleotide levels in erythrocytes, 6-MP dose ratio, and frequency of leukopenia, but *TPMT* and other variants were not associated among Korean children with ALL[9]. In our population, the average 6-MP dose for heterozygous carriers of *TPMT* rs1142345 was similar to wild-type patients, but the average dose for patients with both *TPMT* rs1142345 and *NUDT15* rs116855232 was 50% of tolerable dose of the wild-type patients. Moreover, the standard dose of 6-MP on protocol for childhood ALL in Asian countries[29] is set at 70% of the dose (40-60 mg/m^2) applied to protocols in Europe and US (75 mg/m^2). Although there does appear to be an effect of *TPMT* rs1142345 on 6-MP tolerability in Asians in the presence of *NUDT15* variation, these effects may be small due to low prevalence of the variant and a low standard dose. Results suggest that heterozygous variants of both *NUDT15* and *TPMT* may induce less tolerability for 6-MP than *NUDT15* heterozygous genotype alone.

Other genetic variants identified through candidate gene approaches have been reported to be associated with thiopurine tolerable dose. In our study, *MTHFR* rs1801133 was associated with 6-MP dose, but the direction of association is in contrast to other reports[24]. *MTHFR* is involved in folate metabolism and rs1801133 has been associated with hepatotoxicity. However, our study showed rs1801133 to be associated with high 6-MP average dose. Other previously reported candidate genetic variants were not associated with 6-MP average dose in our study.

In maintenance therapy, 6-MP dose and therapeutic duration is different by country, and allele frequencies of candidate variants may be different. This circumstance makes validation of SNP associations and consistency a challenge. The candidate missense SNP rs61886492 in *FOLH1*could not be evaluated in the current study due to its absence in the Japanese population. This variant was previously reported to be associated with 6-MP mediated toxicity [25]. Interestingly, regional evaluation of genetic variation of this gene in our study identified a significant association signal led by SNP rs12574928 located in an intronic region. This SNP has been described as an expression quantitative trail locus (eQTL) for *FOLH1* in multiple tissues as documented in the Genotype-Tissue Expression (GTEx) Portal. Due to LD broadly spread across this relatively short gene, localizing a defined causal region will require additional studies.

We showed that *NUDT15* rs116855232 is the most prominently associated genetic loci for 6-MP tolerance in Japanese children with ALL. However, 6 of 20 patients who required significant 6-MP dose reduction to a level below 20 mg/m^2/day did not carry the known risk variants for 6-MP tolerance. Moreover, 17 patients needed to increase 6-MP dose to a level greater than 60 mg/m^2/day. Five previously unreported variants located in four genes showed suggestive associations with 6-MP tolerance, including loci residing in *AFF3, CHST11, THADA* and *CMIP*. However, these suggestive variants are located in intronic regions, and their functional significance has not been reported. The associated *AFF3* rs75364948 and surrounding SNPs in strong LD showed to be potential eQTL for

AFF3 and *KIAA1211L*. *AFF3* encodes a tissue-restricted nuclear transcriptional activator, and *AFF3* upregulation was reported to be high in tamoxifen resistant breast cancer[30]. While the function of *KIAA1211L* is not clear, a previous study of molecular markers among childhood leukemia patients showed this gene to be differentially expressed between patients with and without central nervous system involvement[31]. *CHST11* was reported previously in relation to the efficacy of methotrexate treatment in rheumatoid arthritis [32]. *THADA* and *CMIP* genetic variants were identified in GWAS as a susceptibility locus for type 2 diabetes mellitus[33,34], and other diseases[35,36]. These suggestive findings could not be replicated in our small patient series in the present study. Future activities will comprise securing additional patient numbers for a statistically robust opportunity to confirm these findings in further replication attempts.

In this study, 6-MP average dose for initial 168 days of maintenance therapy was negatively correlated with age at diagnosis. Therefore, the association of 6-MP and genetic variant was adjusted by age. Although there have been no reports on the relationship between 6-MP tolerable dose and age, some reports have shown that age was negatively correlated with TPMT activity[37].

There are some limitations of this study to acknowledge. Due the retrospective nature of this study, we decided to exclude patients who started at more than 125% or less than 75% of protocol 6-MP dose. These patients were not suitable for evaluation for 6-MP response to standard therapy in maintenance therapy because therapeutic dose for 6-MP and MTX were adjusted for drug induced toxicities before maintenance therapy. Therefore, it is possible that our study cohort underrepresented patients who deviated from the normal 6-MP tolerance. Despite this limitation, our study is meaningful in describing the responsible genetic factors of 6-MP tolerance among patients starting with standard protocol dose.

Conclusion

We provide strong evidence that *NUDT15* rs116855232 is the prominent genetic factor associated with 6-MP tolerable dose, and it currently represents the most meaningful clinically predictive marker in Japanese. These findings indicated that *NUDT15* genotyping should be considered prior to initiation of 6-MP treatment. Prospects for the identification of additional loci of 6-MP tolerance are high after further validation of suggestive loci observed in this Japanese study.

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Conflict of interest

There are no conflicts of interest to declare.

Authorship contributions

Y.T., K.Y.U., M.T. and A.T designed the experiments. Y.T., K.Y.U. and M.H analyzed data. M.M., Y.A., D.H., Y.N., M.Y., D.K., S.O., K.A., T.I., M.T. K.K., and A.M. collected the clinical data. T.K. and F.M. contributed to perform genotyping. Y.T. and K.Y.U. wrote the paper. All authors approved the manuscript and the interpretation of the data.

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Figure legends

Figure 1. Manhattan plot of results of the GWAS using linear regression adjusting for age at diagnosis.

Figure 2. Average 6-MP dose for 168 days from initial maintenance therapy in the combined Discovery and replication cohort (N = 279). Difference between genotypes were analyzed by the Kruskal-Wallis test. (A) *NUDT15* rs116855232; (B) *AFF3* rs75364948; (C) *CHST11* rs1148407.

Table 1. Demographic and clinical characteristics of patients

	Discovery	Replication
Patient number	224	55
Gender		
Female	99 (44.2%)	24 (43.6%)
Male	125 (55.8%)	31 (56.4%)
Age at diagnosis (years)		
0-1	14 (6.3%)	3 (5.5%)
2-4	104 (46.4%)	19 (34.5%)
5-10	79 (35.3%)	26 (47.3%)
11-19	27 (12.0%)	7 (12.7%)
Median (range)	4.7 (0.9 – 15.7)	5.0 (1 – 17)
6-mercaptopurine initial dose (mg/m^2, median)	39.9 (30.2 - 50.0)	40.1 (30.8 – 49.5)
6-mercaptopurine average dose for 168 days (mg/m^2, median)	41.1 (13.3 – 78.5)	37.2 (5.5 – 60.0)
methotrexate dose (mg/m^2/week, median)	24.5 (0 – 31.5)	24.7 (0 – 30)

Table 2. Results of the genome-wide association analysis of 6-mercaptopurine dose and replication (age adjusted)

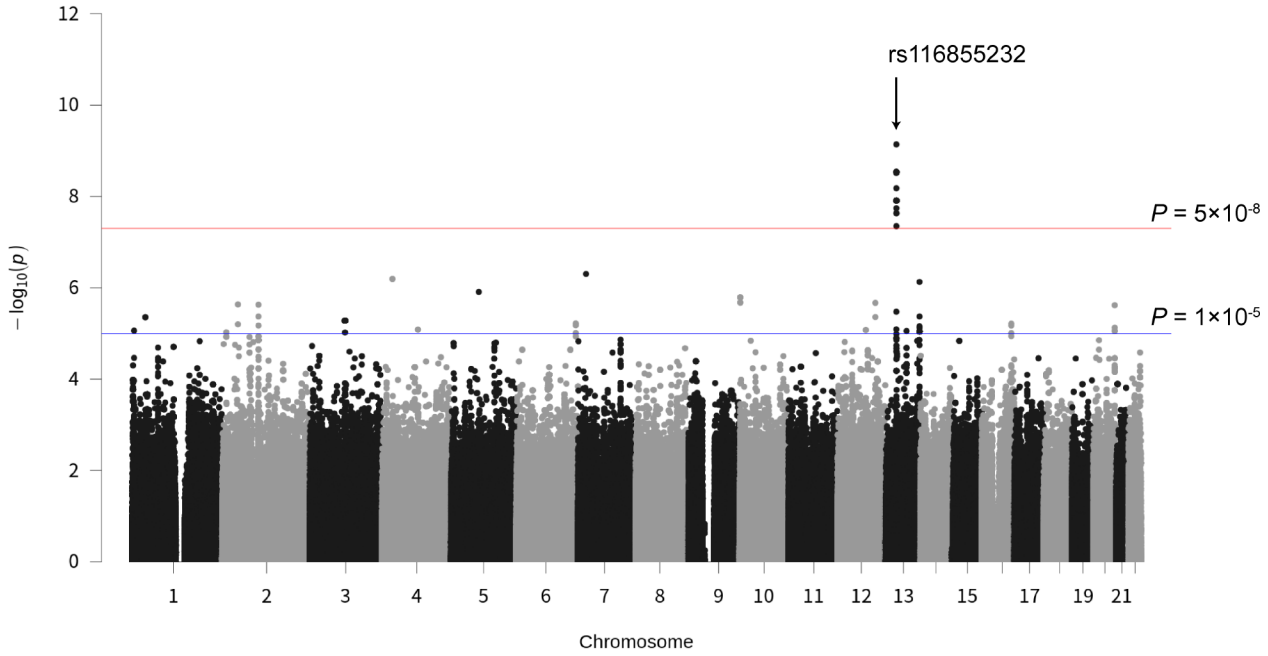
					Discovery (N = 224)		Replication (N = 55)		Combined (N = 279)	
Chr	Position (GRCh37)	Gene	SNP	MAF in discovery	β (95% CI)	P	β (95% CI)	P	β (95% CI)	P
13	48619855	*NUDT15*	rs116855232	0.094	-11.45 (-14.95, -7.95)	8.46×10^{-10}	-9.13 (-13.29, -4.96)	5.43×10^{-5}	-10.99 (-13.82, -8.16)	3.66×10^{-13}
2	100399866	*AFF3*	rs75364948	0.203	6.49 (3.88, 9.10)	2.05×10^{-6}	0.84 (-4.76, 6.44)	0.766	5.83 (3.42, 8.24)	3.12×10^{-6}
12	105058593	*CHST11*	rs1148407	0.138	8.08 (4.83, 11.3)	2.09×10^{-6}	2.22 (-3.28, 7.74)	0.421	6.52 (3.56, 9.49)	2.03×10^{-5}
2	43501170	*THADA*	rs144526347	0.015	21.37 (12.74, 30.00)	2.28×10^{-6}	--	--	--	--
16	81553070	*CMIP*	rs12934986	0.277	5.63 (3.24, 8.01)	6.43×10^{-6}	--	--	--	--
16	81659083	*CMIP*	rs10153053	0.234	-5.91 (-8.40, -3.14)	5.79×10^{-6}	--	--	--	--

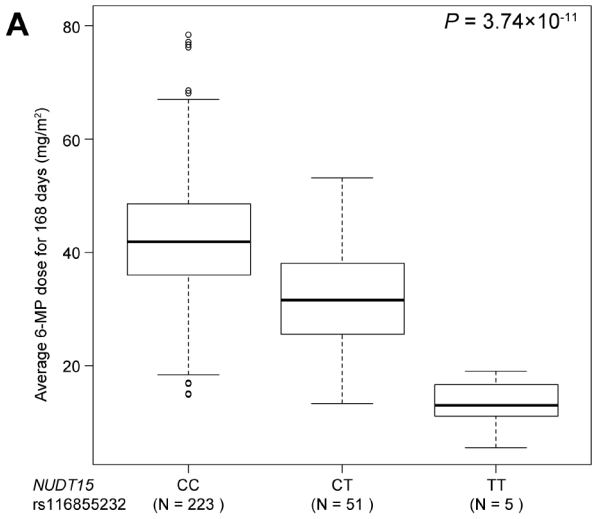
Abbreviations; Chr, chromosome; SNP, single nucleotide polymorphism; MAF, minor allele frequency.

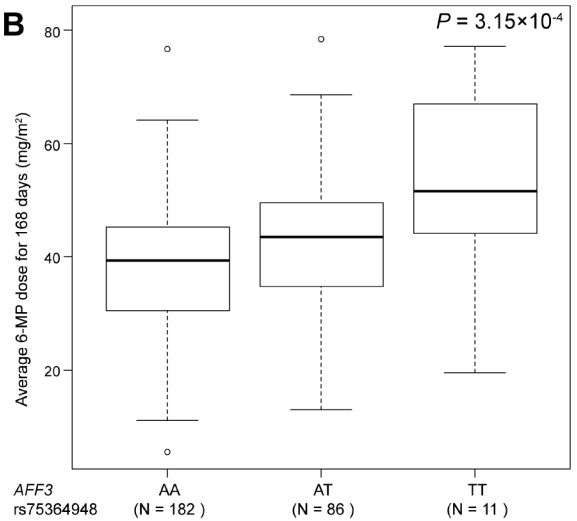
Table 3. Results of the relationship between previously reported candidate genes and average 6-mercaptopurine dose for 168 days (age adjusted)

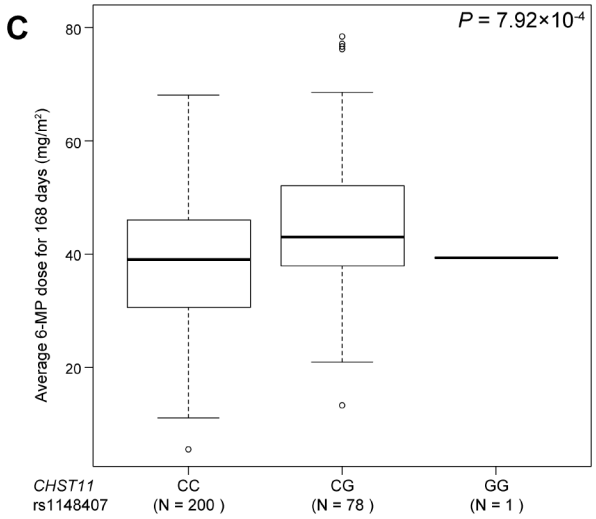
Chr	Position (GRCh37)	Gene	SNP	Function	Alleles	MAF	Discovery (N = 224) β (95% CI)	P (nominal)	P (adjusted)	Reference
Previous reported SNPs										
1	11856378	*MTHFR*	rs1801133	Missense	G/A	0.375	2.26 (0.06, 4.46)	0.0454	--	24
6	18123502	*NHLRC1*	rs12199316	Upstream	C/G	0.394	-0.69 (-3.06, 1.69)	0.553	--	26
6	18130918	*TPMT*	rs1142345	Missense	T/C	0.0156	-2.38 (-11.45, 6.70)	0.608	--	2
10	104878454	*NT5C2*	rs72846714	Intron	G/A	0.024	3.75 (-6.00, 13.5)	0.452	--	27
11	49186274	*FOLH1*	rs61886492	Missense	G/A	--	--	--	--	25
12	21331549	*SLCO1B1*	rs4149056	Missense	T/C	0.147	-2.45 (-5.47, 0.57)	0.113	--	23
13	95815415	*ABCC4*	rs3765534	Missense	C/T	0.143	-1.66 (-4.88, 1.55)	0.310	--	20
20	3193842	*ITPA*	rs1127354	Missense	C/A	0.152	1.01 (-2.05, 4.07)	0.520	--	21, 22
Genetic variants across previously reported candidate gene regions (nominal P-value<0.05)										
11	49213504	*FOLH1*	rs12574928	Intron	C/T	0.056	-8.02 (-12.9, -3.21)	1.59×10^{-3}	0.013	25
13	95707834	*ABCC4*	rs9561773	Intron	C/T	0.228	3.69 (1.02, 6.37)	7.35×10^{-3}	0.577	20
13	95953517	*ABCC4*	rs11568681	Missense	G/T	0.018	10.91 (2.54, 19.29)	0.011	0.707	20
20	3200778	*ITPA*	rs959815466	Intron	C/T	0.013	-11.55 (-21.2, -1.90)	0.019	0.126	21,22

Abbreviations; Chr, chromosome; SNP, single nucleotide polymorphism; MAF, minor allele frequency.


rs116855232
P = 5×10-8
P = 1×10-5
−log10(p)
Chromosome
0
2
4
6
8
10
12
1
2
3
4
5
6
7
8
9
10
11
12
13
15
17
19
21


A
P = 3.74×10-11
Average 6-MP dose for 168 days (mg/m2)
80
60
40
20
NUDT15
rs116855232
CC
(N = 223)
CT
(N = 51)
TT
(N = 5)


B
P = 3.15×10-4
Average 6-MP dose for 168 days (mg/m2)
80
60
40
20
AFF3
rs75364948
AA
(N = 182)
AT
(N = 86)
TT
(N = 11)


C
P = 7.92×10-4
Average 6-MP dose for 168 days (mg/m2)
80
60
40
20
CHST11
rs1148407
CC
(N = 200)
CG
(N = 78)
GG
(N = 1)